(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 158 487 B1**

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication of patent specification: **28.08.91** (51) Int. Cl.5: **A61K 37/02**

(21) Application number: **85302176.4**

(22) Date of filing: **28.03.85**

The file contains technical information submitted after the application was filed and not included in this specification

(54) Stable composition of interleukin-2.

(30) Priority: **09.04.84 JP 71568/84**
25.01.85 JP 13226/85
25.02.85 JP 37184/85

(43) Date of publication of application:
16.10.85 Bulletin 85/42

(45) Publication of the grant of the patent:
28.08.91 Bulletin 91/35

(84) Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

(56) References cited:
EP-A- 0 091 258
EP-A- 0 145 390
FR-A- 2 532 178

JOURNAL OF IMMUNOLOGY, vol. 128, no. 3, March 1982, pages 1122-1127, the American Association of Immunologists, US; J.W. MIER et al.: "The purification and properties of human T cell growth factor"

(73) Proprietor: **TAKEDA CHEMICAL INDUSTRIES, LTD.**
3-6, Doshomachi 2-chome Chuo-ku
Osaka(JP)

(72) Inventor: **Mikura, Yasushi**
30-11, Senriyamahigashi 1-chome
Suita Osaka 565(JP)
Inventor: **Asada, Kensuke**
A13-201. 23 Yamadanishi 1-chome
Suita Osaka 565(JP)
Inventor: **Toguchi, Hajime**
7-20, Koushienguchi 3-chome
Nishinomiya Hyogo 663(JP)

(74) Representative: **Laredo, Jack Joseph et al**
Elkington and Fife Beacon House 113 Kingsway
London, WC2B 6PP(GB)

EP 0 158 487 B1

Description

This invention relates to an interleukin 2 composition useful as a drug.

Interleukin-2 (hereinafter sometimes referred to by the abbreviation IL-2) is a protein capable of functioning as a growth factor for T cells and natural killer cells which are considered to play an important role in in vivo immunomodulation and directly or indirectly contribute to elimination of cancer or recovery from or improvement in the immunocompromised state [Nature, 302, 305-310 (1983)]. Having such physiological activity, IL-2 is much expected to be usable as a novel type of anticancer agent or a therapeutic agent for immunodeficiency.

The present inventors found that IL-2 is unstable and easily loses its activity during the process of freezing or lyophilization and during storage following lyophilization, in particular in the step of drying in lyophilization and that solutions obtained upon redissolution of lyophilized IL-2 preparations generally assume turbidity. These facts, among others, are very unfavorable for the use of IL-2 for therapeutic purposes.

Under these circumstances, the present inventors conducted further intensive investigations and succeeded in producing a stable IL-2 composition, and have now completed the present invention.

The present invention provides an IL-2 composition which comprises human serum albumin, a reducing compound or a combination thereof and is adjusted to a pH of 3 to 6 as a solution.

In the practice of the invention, the IL-2 may be of any mammal origin but is preferably of human origin. The IL-2 may also be a natural one or a product of the recombinant DNA technology, although the latter is preferred. It is generally used in the form of an aqueous solution of IL-2.

Preferred examples of IL-2 are non-glycosylated human IL-2 species produced by genetic engineering and having the formula:

X-Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
Gln Leu Glu His Leu Leu Leu Asp^{20} Leu Gln Met Ile Leu
Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg
Met Leu^{40} Thr Phe Lys Phe Try Met Pro^{60} Lys Lys Ala Thr
Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys
Pro Leu Glu^{80} Glu Val Leu Asn Leu Ala Gln Ser Lys Asn
Phe His Leu Arg Pro Arg Asp Leu Ile Ser^{100} Asn Ile Asn
Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe
Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu
Phe Leu Asn Arg^{120} Trp Ile Thr Phe Cys Gln Ser Ile Ile
Ser Thr Leu Thr^{133} (I)

wherein X is Met or hydrogen. Mixtures of these may also be used.

In the above formula (I), each amino acid residue is represented by the abbreviation according to the IUPAC-IUB Commission on Biochemical Nomenclature.

The IL-2 desirably has a specific activity of 20,000 to 80,000 units/mg and is advantageously used in the form of an aqueous IL-2 solution having a concentration of 1 to 80,000 units/ml, preferably 10 to 50,000 units/ml; more preferably 50 to 5,000 units/ml. The above aqueous IL-2 solution as the raw material in the practice of the invention is preferably free from salts such as sodium chloride. When said solution is contaminated with a salt in the IL-2 purification process, for instance, it is preferable to remove the salt by ultrafiltration, for instance, prior to use thereof.

The human serum albumin (hereinafter referred to by the abbreviation HSA) may be of any grade. For clinical application of the composition according to the invention, however, said HSA is preferably of a quality allowing the use by parenteral administration.

For instance, there is used HSA fractionated and purified by Cohn's 6th method of ethanol fractionation with healthy human plasma as the starting material [J. Am. Chem. Soc. 68, 459-475 (1946)].

Said HSA may also contain acetyltryptophan sodium or sodium caprylate as a stabilizer.

The addition level of HSA is preferably 0.1 to 50 mg, more preferably 0.5 to 20 mg, per milliliter of the

aqueous IL-2 solution having an IL-2 concentration within the range mentioned above.

The reducing compound is preferably a physiologically acceptable reducing compound and thus includes sulfur-containing reducing compounds such as glutathione (reduced form; hereinafter simply appreciated glutathione), thioctic acid, N-acetylcysteine, N-acetylhomocysteine, thiodiglycol, thioethanolamine, monothioglycerol, dithiothreitol and thioalkanoic acids containing 1-7 carbon atoms (e.g. thioglycolic acid, thiomalic acid), and ascorbic acid and salts thereof, amongst others. Preferred are acidic compounds such as glutathione, thioctic acid, N-acethylcysteine and ascorbic acid, and particularly preferred are glutathione and ascorbic acid.

The reducing compounds mentioned above may be used either alone or in combination of two or more.

These reducing compounds are used preferably in an amount of not less than 0.01 mg, more preferably 0.05 to 20 mg, per milliliter of the aqueous IL-2 solution having a concentration within the above-mentioned range.

HSA and the reducing compound mentioned above may be used both of them within the above-mentioned range or each of them alone, and HSA is preferably used.

The IL-2 composition according to the invention may further contain one or more substances selected from among amino acids, in particular monoamino aliphatic amino acids and cyclic amino acids, such as glycine, glutamic acid, aspartic acid, alanine and proline, monosaccharides such as glucose and mannose, sugar alcohols such as sorbitol and mannitol, and physiologically acceptable salts and derivatives thereof. Among these auxiliary additives, glycine is particularly preferred.

The above auxiliary additives are preferably used in amounts of 10-100 mg for monosaccharides and sugar alcohols and 5-50 mg for amino acids per milliliter of the above-mentioned aqueous IL-2 solution.

The IL-2 composition according to the invention may further contain an isotonizing agent such as sodium chloride, a buffer such as succinic acid, tartaric acid or citric acid, and/or a surfactant. However, the IL-2 composition is preferably free of sodium chloride from the viewpoint of stabilization in the process of lyophilization.

In order that the IL-2 composition according to the present invention gives a pH of 3 to 6, preferably 3 to 5.5, more preferably 3.5 to 4.5, said composition is adjusted to a pH within the range specified herein with an acidic reducing compound or an acidic amino acid such as glutamic acid when such compound is added, or, in cases when if further desired and when no acidic compound is contained, with a mineral acid such as hydrochloric acid or phosphoric acid, or a buffer of organic acid such as succinic acid, tartaric acid or citric acid.

The stability of the above IL-2 composition may be further increased by evacuating the space within containers for the IL-2 composition or filling said space with nitrogen.

The IL-2 composition according to the invention preferably takes the form of an aqueous solution, or frozen matter, or lyophilizate , in particular the form of a lyophilizate.

The composition according to the invention may be produced, for example in the following manner:

To an aqueous solution of IL-2 in a concentration of 1 to 80,000 units/ml, there is added HSA or/and reducing compound to a predetermined concentration, followed by pH adjustment in the manner mentioned above.

Monosaccharides, sugar alcohols and amino acids, for instance, may also be added in the respective concentrations mentioned above. If desired, an isotonizing agent, and a surfactant may further be added. When some substance other than HSA is added, pH adjustment is performed in the manner mentioned above so that the final aqueous solution can have a pH within the above range. The IL-2 composition thus obtained may be used also as the raw material in producing a frozen matter or a lyophilizate in the manner mentioned below.

The frozen form of the IL-2 composition may be produced, for example, by freezing the above aqueous solution generally at -80° to -20° C. Said frozen composition is preferably stored at -80° to -10° C.

The lyophilizate form of the IL-2 composition may be produced, for example, by drying the above frozen composition under reduced pressure in the conventional manner or by freezing the above aqueous solution or an aqueous solution resulting from thawing the above frozen composition, in the same manner as above, following distribution thereof as desired, and then drying the resulting frozen composition under reduced pressure by the conventional method.

Furthermore, the IL-2 composition according to the invention which is in the form of a solution may be produced by redissolving a lyophilizate containing IL-2, HSA or/and a reducing compound or/and a pH adjusting agent as produced by the method mentioned above in a solvent containing a monosaccharide, sugar alcohol or amino acid and pH-adjusted, for example, with hydrochloric acid, as desired.

In producing the lyophilized IL-2 composition according to the invention as an injectable preparation, it is preferable to combine the IL-2-containing aqueous solution with an additive-containing aqueous solution,

each after separate sterile filtration, or purify a mixture of the IL-2-containing aqueous solution and an additive-containing aqueous solution by sterile filtration , then distribute the mixture aseptically into vials and subject the mixture in vials to the above-mentioned lyophilization treatment.

In dissolving the lyophilizate in an aqueous solution containing an amino acid, monosaccharide or sugar alcohol, it is preferred that the aqueous solution is subjected to sterile filtration, then distributed into ampuls and autoclaved prior to its use as the solvent.

The IL-2 composition according to the invention is advantageously characterized, among others, in that the decrease in IL-2 activity during storage and freezing and lyophilization procedures is minimum and that when it occurs as a lyophilizate, the solution obtained upon redissolution thereof is clear and transparent.

The IL-2 composition according to the invention, in particular the one in the form or lyophilizate, has an improved appearance and the adsorption thereof on the container wall is effectively prevented.

The composition which also contains an amino acid, when lyophilized, assumes an improved appearance. The pain at the time of administration thereof by injection is also effectively alleviated.

The monosaccharide-containing composition also attains a pain-alleviating effect at the time of administration thereof by injection.

Among those IL-2 compositions provided by the invention, the lyophilizate form may be obtained as a stabilized IL-2 powder and may be used advantageously as a preparation for parenteral administration, among others. In using as a preparation for injection, the lyophilizate is dissolved in 0.5-100 ml of distilled water for injection or physiological saline, for instance, or in 0.5-100 ml of a solvent attached to the lyophilizate composition for exclusive use therefor, which is an aqueous solution of an amino acid such as glycine, a monosaccharide such as glucose or a sugar alcohol such as mannitol as pH-adjusted as necessary, and the solution is administered intramuscularly or intravenously. Said composition may also be used in the form of preparations for administration into the oral or nasal cavity or to the eye or ear as made up by using an appropriate carrier, excipient or diluent.

The IL-2 composition according to the invention is low in toxicity and may be used for the same purposes and in the same manner as the known IL-2 preparations, in particular for use in the production of an anticancer agent or a therapeutic agent for immunodeficiency.

The IL-2 activity data given in units (U) in the present specification were obtained in the following manner:

Thus, an IL-2-containing test sample was added to a suspension, in a medium, of a mouse cell line capable of growing in an IL-2 concentration-dependent manner. After incubation, the growth of said cell line was determined with the uptake of tritiated thymidine as an index. In assaying, a standard IL-2 (1 U/ml) was always used in parallel with the test sample and the activity in units (U) of the test sample was calculated from the activity ratio therebetween.

Specifically, an IL-2-dependent mouse cell line [NKC3; Hinuma et al., Biochemical and Biophysical Research Communications, 109, 363 (1982)] maintained by subculture in RPMI 1640 medium containing human IL-2-containing conditioned medium plus 20% FCS (fetal calf serum) at 37° C in the presence of 5% CO_2 was used. The cells were washed twice with serum-free RPMI 1640 medium and resuspended in 20% FCS-added RPMI 1640 medium in a concentration of 6×10^5 cells/ml.

All IL-2-containing test sample was distributed, in 50 μl portions, into the first row of wells on a 96-well flat-bottomed microtiter plate (Nunc, Denmark), followed by serial doubling dilution to the 12th row using 50 μl of 20% FCS-added RPMI 1640 medium per well. Then, 50 μl of the above NKC3 cell suspension was added to each well. Incubation was conducted at 37° C in the presence of 5% CO_2 for 24 hours, during which, at 20 hours of incubation, 1 μCi of tritiated thymidine (Amersham, Great Britain) was added to each well. Cells were recovered on a glass filter using a cell harvester (Flow, U.S.A.) and measured for tritiated thymidine uptake using a liquid scintillation counter. In parallel, the same procedure was followed with a standard IL-2 sample for measuring tritiated thymidine uptake.

Activity calculation in units (U) was performed by the profit method according to Journal of Immunology, 120, 2027 (1978). Thus, among a dilution series derived from a standard IL-2 sample (the culture supernatant after 48-hour incubation, at 37° C in the presence of 5% CO_2, of a 5×10^5/ml suspension of human peripheral blood lymphocytes in 10% FCS added RPMI 1640 medium with 40 μg of concanavalin A and 15 ng/ml of 12-O-tetradecanoylphorbol-13-acetate added being defined as having an activity of 1 U/ml), the maximum uptake was taken as 100%, and the percentage uptake (%) for each dilution stage was calculated. The values obtained were plotted on a normal probability paper and the dilution factor corresponding to 50% uptake was determined graphically. For each IL-2-containing test sample, the dilution factor corresponding to 50% uptake was determined in the same manner.

The IL-2 concentration (U/ml) in the test sample was calculated using the formula:

Dilution factor at which test samples shows 50% uptake

Dilution factor at which standard IL-2 sample shows 50% uptake

The transformant Escherichia coli DH1/pTF4 disclosed hereinafter in Reference Example has been deposited with the Fermentation Institute, Osaka under the deposit number IFO-14299 and, since April 6, 1984, with the Fermentation Research Institute (FRI), Agency of Industrial Science and Technology, Ministry of International Trade and Industry under the deposit number FERM BP-628.

Examples

The following working examples and reference example illustrate the invention in further detail. However, they are by no means limitative of the present invention.

The stock solution used in the working examples was the non-glycosylated human IL-2 protein solution obtained by the method described in the reference example.

Example 1

To an aqueous solution (0.5 ml) containing 2,450 units of human IL-2 as prepared by dilution of the stock solution with distilled water for injection followed by sterile filtration, there was added a solution (0.5 ml) containing 10 mg of glutathione or ascorbic acid after sterile filtration. The two aqueous solutions (each 1 ml) thus obtained (pH 3.4 and pH 3.5, respectively) were each placed in a vial, frozen at -40° C, and lyophilized. Thereafter, the free space in the vial was filled with gaseous N_2 and the vial was stoppered tightly.

The same amount of an aqueous solution free of glutathione or ascorbic acid and the same amount of an aqueous solution containing 25 mg of mannitol, which is in frequent use in lyophilized preparations, in place of glutathione or ascorbic acid were used as controls and lyophilized in the same manner.

These lyophilizates were each redissolved in 1 ml of distilled water for injection and the solutions were examined for solubility (clarity) and potency. As for the potency, the potency of the aqueous solution prior to lyophilization was taken as 100% and the residual percentage was calculated. As the results shown in Table 1 indicate, the IL-2 compositions according to the invention were significantly superior both in solubility and residual potency to the controls.

Table 1

Additive (mg)		Solubility	Residual potency
None		Turbid	47%
Mannitol	(25)	Turbid	58%
Glutathione	(10)	Clear	97%
Ascorbic acid	(10)	Clear	100%

Example 2

To an aqueous solution (0.5 ml) containing 7,680 units of human IL-2 as prepared by dilution of the stock solution with distilled water for injection followed by sterile filtration, there was added a solution (0.5 ml) containing 2 mg of glutathione or ascorbic acid after sterile filtration. The two aqueous solution (each 1 ml) thus obtained (pH 3.6 and pH 3.7, respectively) were lyophilized in the same manner as in Example 1 and the lyophilizates were examined for solubility directly after manufacture and solubility and residual potency after storage at 25° C for 1 month.

The results obtained are shown in Table 2.

Table 2

Additive (mg)	Directly after manufacture	After 1 month at 25°C	
		Solubility	Potency
Glutathione (2)	Clear	Clear	99%
Ascorbic acid (2)	Clear	Clear	90%

Example 3

To an aqueous solution (0.5 ml) containing 7,680 units of human IL-2 as prepared by dilution of the stock solution with distilled water for injection followed by sterile filtration, there was added a solution (0.5 ml) containing 5 ml of HSA plug 2 mg of glutathione or ascorbic acid after sterile filtration. The two aqueous solutions (each 1 ml) thus obtained (pH 4.1 and pH 4.2, respectively), were lyophilized in the same manner as in Example 1 and the lyophilizates were examined for solubility and residual potency in the same manner as in Example 2.

The results obtained are shown in Table 3.

Table 3

Additive (mg)	Directly after manufacture	After 1 month at 25°C	
	Solubility	Solubility	Potency
Glutathione (2) plus HSA (5)	Clear	Clear	104%
Ascorbic acid (2) plus HSA (5)	Clear	Clear	101%

Example 4

To an aqueous solution (0.5 ml) containing 7,680 units of human IL-2 as prepared by dilution of the stock solution with distilled water for injection followed by sterile filtration, there was added a solution (0.5 ml) containing 5 mg of HSA, 9 mg of sodium chloride and 2 mg of glutathione or ascorbic acid after sterile filtration. The two aqueous solutions (each 1 ml) thus obtained (pH 4.1 and pH 4.2, respectively) were lyophilized in the same manner as in Example 1 and the lyophilizates were examined for solubility and residual potency in the same manner as in Example 2.

The results obtained are shown in Table 4.

Table 4

Additive (mg)	Directly after manufacture	After 1 month at 25°C	
	Solubility	Solubility	Potency
Glutathione (2) + HSA (5) + sodium chloride (9)	Clear	Clear	85%
Ascorbic acid (2) + HSA (5) + sodium chloride (9)	Clear	Clear	93%

Example 5

To an aqueous solution (0.5 ml) containing 7,680 units of human IL-2 as prepared by dilution of the stock solution with distilled water for injection followed by sterile filtration, there was added a solution (0.5 ml) containing 50 mg of mannitol and 2 mg of glutathione or ascorbic acid after sterile filtration. The two aqueous solutions (each 1 ml) thus obtained (pH 3.4 and pH 3.6, respectively) were lyophilized in the same manner as in Example 1 and the lyophilizates were examined for solubility and residual potency in the same manner as in Example 2.

The results obtained are shown in Table 5.

Table 5

Additive (mg)	Directly after manufacture Solubility	After 1 month at 25°C Solubility	Potency
Glutathione (2) + mannitol (50)	Clear	Clear*	114%
Ascorbic acid (2) + mannitol (50)	Clear	Clear	95%

* Data after storage at 25°C for 6 days.

Example 6

To an aqueous solution (0.5 ml) containing 23,350 units of human IL-2 as prepared by dilution of the stock solution with distilled water for injection followed by sterile filtration, there was added an aqueous solution (0.5 ml) containing 5 mg of glutathione and 23 mg of glycine after sterile filtration. The aqueous solution (1 ml) thus obtained (pH 3.7) was lyophilized in the same manner as in Example 1 and the lyophilizate was examined for solubility and residual potency directly after manufacture and after storage at 40° C for 3 weeks in the same manner as in Example 1.

The results obtained are shown in Table 6.

Table 6

Additive (mg)	Directly after manufacture Solubility	Potency	After 1 month at 40°C Solubility	Potency
Glutathione (2) + glycine (23)	Clear	95.1%	Clear	107.1%

Example 7

To an aqueous solution (0.5 ml) containing 1,790 or 130 units of human IL-2 as prepared by dilution of the stock solution with distilled water for injection followed by sterile filtration, there was added an aqueous solution (0.5 ml) containing 2 mg of glutathione, 5 mg of HSA and 9 mg of sodium chloride after sterile filtration. The two aqueous solutions (each 1 ml) thus obtained (pH 3.9 both) were lyophilized in the same manner as in Example 1 and the lyophilizates were examined for solubility and residual potency directly after manufacture and after storage at 40° C for 1 week in the same manner as in Example 1.

The results obtained are shown in Table 7.

Table 7

Human IL-2 (units)	Additive (mg)	Directly after manufacture Solubility	Potency	After 1 week at 40°C Solubility	Potency
1,790	Glutathione (2) + HSA(5) + sodium chloride (9)	Clear	90%	Clear	94%
130	Same as above	Clear	94%	Clear	89%

Example 8

To an aqueous solution (0.5 ml) containing 1,860 or 116 units of human IL-2 as prepared by dilution of the stock solution with distilled water for injection followed by sterile filtration, there was added an aqueous solution (0.5 ml) containing 2 mg of glutathione, 1 mg of HSA and 23 mg of glycine after sterile filtration. The two aqueous solutions (each 1 ml; pH 3.8 and pH 3.9, respectively) were lyophilized in the same manner as in Example 1 and the lyophilizates were examined for solubility and residual potency directly after manufacture and after storage at 40° C for 1 week.

The results obtained are shown in Table 8.

Table 8

Human IL-2 (units)	Additive (mg)	Directly after manufacture Solubility	Potency	After 1 week at 40°C Solubility	Potency
1,860	Glutathione (2) + HSA (1) + glycine (23)	Clear	90%	Clear	98%
116	Same as above	Clear	110%	Clear	108%

Example 9

To an aqueous solution (0.5 ml) containing 17,600 units of human IL-2 as prepared by dilution of the stock solution with distilled water for injection followed by sterile filtration, there was added an aqueous solution (0.5 ml) containing 5 mg of HSA and having a pH of 4 (adjusted with hydrochloric acid, after sterile filtration or an aqueous solution (0.5 ml) containing 5 mg of HSA and 9 mg of sodium chloride and having a pH of 4 (adjusted with hydrochloric acid) after sterile filtration. The two aqueous solutions (each 1 ml) thus obtained were each placed in a vial, frozen at -40° C, and lyophilized. Thereafter, the free space in the vial was filled with gaseous N_2 and the vial was stoppered tightly.

These lyophilizates were each redissolved in 1 ml of distilled water for injection directly after manufacture or after storage at 40° C for 0.5 month and the solutions were examined for solubility (clarity) and potency. As for the potency, the potency of the aqueous solution prior to lyophilization was taken as 100% and the residual percentage was calculated based thereon. As the results shown in Table 9 indicate, the IL-2 compositions according to the invention were significantly superior in solubility and residual potency.

Table 9

Additive (mg)	Directly after manufacutre		After 0.5 month at 40°C	
	Solubility	Potency	Solubility	Potency
HSA (5) pH-adjusted with hydrochloric acid	Clear	102.3%	Clear	100.6%
HSA (5) + sodium chloride (9), pH-adjusted with hydrochloric acid	Clear	113.6%	Clear	110.2%

Example 10

To an aqueous solution (0.5 ml) containing 4,115 units/ml of human IL-2 as prepared by dilution of the stock solution with distilled water for injection followed by sterile filtration, there was added an aqueous solution (0.5 ml) containing 5 mg of HSA, 5 mg of HSA plus 9 mg of sodium chloride, 5 mg of HSA plus 23 mg of glycine, or 5 mg of HSA plus 50 mg of mannitol and having a pH of 4.0 (adjusted with hydrochloric acid) after sterile filtration. The four kinds of aqueous solutions (each 1 ml) thus obtained were each placed in a vial, frozen at -40° C, and lyophilized. Thereafter, the vial space was filled with gaseous N_2 and each vial was stoppered tightly. As controls, an aqueous solution of human IL-2 alone and various aqueous IL-2 solutions containing no pH adjusting agent were used in the same amount and lyophilized in the same manner as above.

These lyophilizates were examined for appearance and thereafter each redissolved in 1 ml of distilled water for injection, 0.9% physiological saline, 5% aqueous glucose solution, 5% aqueous sorbitol solution, or 5% aqueous mannitol solution, and the solutions were examined for pH and solubility (clarity).

As the results shown in Table 10 indicate, the IL-2 compositions according to the invention were significantly superior in solubility to the controls. In particular, the lyophilizate with HSA and glycine incorporated therein, which gave a pH of about 4, was superior in solubility.

Table 10

Human IL-2: 2058 units

Additive (mg)	pH adjuster	Appearance	Solvent for redissolution	pH	Solubility	Remarks
None	None	Bad	Distd. water for injection	5.6	Turbid	Control
None	HCl*	"	"	4.2	"	Control
HSA (5)	None	Good	"	5.8	"	Control
"	HCl*	"	"	4.3	Clear	
"	"	"	0.9% physiol. saline	4.3	"	
"	"	"	5% glucose aq.	4.3	"	
"	"	"	5% sorbitol aq.	4.3	"	
"	"	"	5% mannitol aq.	4.3	"	
"	"	"	2.3% glycine aq.	4.1	"	Solvent adjusted to pH 4.1
HSA(5) NaCl(9)	None	"	Distd. water for injection	6.3	Turbid	Control
"	HCl*	"	"	3.9	Clear	
HSA(5) glycine(23)	None	"	"	6.3	Turbid	Control
"	HCl*	"	"	3.9	Very clear	
HSA(5) mannitol(50)	None	"	"	5.6	Turbid	Control
"	HCl*	"	"	4.1	Clear	

*Hydrochloric acid

Example 11

Two aqueous solutions (each 1 ml) obtained by the procedure of Example 9 and deprived of bacteria by filtration and containing 1,620 or 128 units of human IL-2, 5 mg of HSA and 23 mg of glycine and having

a pH of 4.0 (adjusted with hydrochloric acid) were lyophilized in the same manner as in Example 9 and the lyophilizates were examined for solubility and residual potency directly after manufacture and after storage at 40° C for 1, 2 and 4 weeks in the same manner as in Example 9.

The results obtained are shown in Table 11.

Table 11

Human IL-2 (units)	Additive (mg)	Directly after manufacture		After 1 week at 40°C		After 2 weeks at 40°C		After 4 weeks at 40°C	
		pH	Solubility	Solubility	Residual potency	Solubility	Residual potency	Solubility	Residual potency
1620	HSA(5) + glycine (23) pH-adjusted with HCl	4.0	Very clear	Very clear	101.2%	Very clear	101.9%	Very clear	98.8%
128	Same as above	4.0	Very clear	Very clear	100.8%	Very clear	101.6%	Very clear	100.0%

Example 12

To an aqueous solution (0.5 ml) containing 2,450 units of human IL-2 as prepared by dilution of the stock solution with distilled water for injection followed by sterile filtration, there was added distilled water for injection (0.5 ml), a sterile filtrate (0.5 ml) containing 10 mg of glutathione, glutathione disodium salt, ascorbic acid or sodium ascorbate or a sterile filtrate (0.5 ml) adjusted to acidic condition with hydrochloric acid and containing 10 mg of glutathion disodium salt or sodium ascorbate. The 7 kinds of aqueous solutions thus obtained were each placed in a vial, frozen at -40°C, and lyophilized. Thereafter, the free space in the vial was filled with gaseous N_2 and the vial was stoppered tightly. These lyophilizates were each redissolved in 1 ml of distilled water for injection and the solutions were examined for pH and solubility (clarity). As the results shown in Table 12 indicate, the IL-2 compositions according to the invention were significantly superior in solubility to the controls.

Table 12

Additive (mg)	pH adjuster	pH	Solubility	Remarks
None	None	5.4	Turbid	Control
Glutathione (10)	None	3.4	Clear	
Glutathione disodium(10)	None	9.2	Turbid	Control
Glutathione disodium(10)	HCl*	4.2	Clear	
Ascorbic acid (10)	None	3.5	Clear	
Sodium ascorbate (10)	None	6.5	Some turbid	Control
Sodium ascorbate (10)	HCl*	4.3	Clear	

* hydrochloric acid

Reference Example

Production of Non-glycosylated human IL-2 protein solution

(i) The human IL-2 gene-bearing transformant Escherichia coli (E. coli) DH1/pTF4 obtained in Example 3 of the specification for EPC Patent Application No. 84308153.0 was inoculated into 50 ml of a liquid medium (pH 7.0) containing 1% Bacto-tryptone (Difco Laboratories, U.S.A.), 0.5% Bacto yeast extract (Difco Laboratories, U.S.A.), 0.5% sodium chloride and 7 μg/ml tetracycline in a 250-ml erlenmeyer flask, followed by incubation at 37°C overnight in the manner of rolling shake culture. The culture fluid was transferred to a 5-liter jar fermenter containing 2.5 liters of M9 medium containing 0.5% casamino acids, 0.5% glucose and 7 μg/ml tetracycline, followed by 4 hours of incubation at 37°C with aeration and stirring, then addition of 3-8-indolylacrylic acid (25 μg/ml) and a further 4 hours of incubation under the same conditions. The thus-obtained culture fluid (2.5 liters) was subjected to centrifugation and the cells collected were frozen at -80°C and stored.

(ii) The cells obtained in the above (i) and stored in the frozen state (37.5 g) were suspended uniformly in 500 ml of an extractant (pH 7.0) containing 7 M guanidine hydrochloride and 0.1 M Tris•HCl, and the suspension was stirred at 4°C for 1 hour. The resultant lysate solution was centrifuged at 28,000 x g for 20 minutes, giving 453 ml of a supernatant.

(iii) The supernatant obtained in the above (ii) was dialyzed against 0.01 M Tris•HCl buffer (pH 8.5) and then centrifuged at 19,000 x g for 10 minutes. The supernatant thus obtained (458 ml) was applied to a DE52 (DEAE-cellulose, Whatman, Great Britain) column (50 ml in volume) equilibrated in advance with 0.01 M Tris•HCl buffer (pH 8.5) to thereby effect protein adsorption. IL-2 was eluted by constructing a linear NaCl concentration gradient (0 to 0.15 M NaCl, 1 liter). Active fractions (105 ml) were combined, concentrated to 10.2 ml using a YM-5 membrane (Amicon, U.S.A.) and subjected to gel filtration using a Sephacryl S-200 (Pharmacia, Sweden) column (500 ml in volume) equilibrated with 0.1 M Tris•HCl (pH

8.0)-1 M NaCl buffer. Active fractions (56 ml) were concentrated to 4.9 ml with a YM-5 membrane. The concentrate obtained was subjected to high performance liquid chromatography using an Ultrapore RPSC (Altex, U.S.A.) column and a tri-fluoroacetic acid-acetonitrile eluent system.

Column, Ultrapore RPSC (4.6 x 75 cm); column temperature, 30 °C; Eluent A, 0.1% trifluoroacetic acid-99.9% water; eluent B, 0.1% trifluoroacetic acid-99.9% acetonitrile; elution program, minute 0 (68% A + 32% B) -minute 25 (55% A + 45% B) -minute 35 (45% A + 55% B) -minute 45 (30% A + 70% B) -minute 48 (100% B); rate of elution, 0.8 ml/minute; detection wavelength, 230 nm. Active fractions exhibiting a retention time of about 39 minutes under the above conditions were collected and there was obtained 15 ml of a solution containing 7.5 mg of non-glycosylated human IL-2 protein [specific activity, 30,000 U/mg; activity recovery rate from starting material, 48.2%; purity of protein, 99% (as determined by densitometry)-].

Claims

1. An interleukin-2 composition which comprises human serum albumin, a reducing compound or a combination thereof and is adjusted as showing pH of 3 to 6 as a solution.

2. The composition according to Claim 1, wherein the interleukin-2 is human interleukin-2.

3. The composition according to Claim 1 or 2, wherein the interleukin-2 is a recombinant interleukin-2.

4. The composition according to Claim 2, wherein the human interleukin-2 is a non-glycosylated human interleukin-2.

5. The composition according to Claim 1, wherein the interleukin-2 has a specific activity of 20,000 to 80,000 units/mg.

6. The composition according to Claim 1, wherein the interleukin-2 is in a concentration of 1 to 80,000 units/ml as an aqueous solution.

7. The composition according to Claim 1, which is free from a salt.

8. The composition according to Claim 1, which comprises the human serum albumin.

9. The composition according to Claim 8, wherein the human serum albumin is in a concentration of 0.1 to 50 mg/ml as an aqueous solution.

10. The composition according to Claim 1, which comprises the reducing compound.

11. The composition according to Claim 10, wherein the reducing compound is an acidic reducing compound.

12. The composition according to Claim 11, wherein the acidic reducing compound is glutathione, thioctic acid, N-acetylcysteine,thioalkanoic acid of 1 to 7 carbon atoms or ascorbic acid.

13. The composition according to Claim 10, wherein the reducing compound is in a concentration of 0.05 - 20 mg/ml as an aqueous solution.

14. The composition according to Claim 1, which comprises a combination of the human serum albumin and the reducing compound.

15. The composition according to Claim 1, which further comprises an monoamino aliphatic amino acid, a cyclic amino acid, a monosaccharide, a sugar alcohol or a combination thereof.

16. The composition according to Claim 1, which further comprises monoamino aliphatic amino acid.

17. The composition according to Claim 16, wherein the monoamino aliphatic amino acid is in a concentration of 5 to 50 mg/ml as an aqueous solution.

18. The composition according to Claim 1, wherein the composition is adjusted by an acidic reducing compound, an acidic amino acid, a mineral acid or/and a buffer of organic acid.

19. The composition according to Claim 1, wherein the pH is 3 to 5.5.

20. The composition according to Claim 1, which is in a form of an aqueous solution, frozen matter or lyophilizate.

21. The composition according to Claim 1, which is in a form of lyophilizate.

22. A method of producing an interleukin-2 composition comprising human serum albumin, a reducing compound or a combination thereof and being adjusted as showing pH of 3 to 6 as a solution, which comprises incorporating human serum albumin, a reducing compound or a combination thereof into an aqueous solution of interleukin-2 and adjusting the solution as showing PH of 3 to 6.

23. The method according to Claim 22, wherein the resulting solution is further frozen to obtain a frozen composition.

24. The method according to Claim 22, wherein the resulting solution is further lyophilized to obtain a lyophilized composition.

25. The method according to Claim 22, wherein the aqueous solution is adjusted by an acidic reducing compound, an acidic amino acid, a mineral acid or a buffer or organic acid.

26. The method according to Claim 25, wherein the aqueous solution is adjusted by the mineral acid.

27. The method according to Claim 25, wherein the aqueous solution is adjusted by the acidic reducing compound.

28. An interleukin-2 composition according to claim 1 for use in the production of a novel type of anticancer agent or a therapeutic agent for immunodeficiency.

Revendications

1. Composition d'interleukine-2, qui comprend de l'albumine sérique humaine, un composé réducteur ou une combinaison de celui-ci, et qui est ajustée de façon à présenter, en solution, un pH de 3 à 6.

2. Composition selon la revendication 1, dans laquelle l'interleukine-2 est l'interleukine-2 humaine.

3. Composition selon la revendication 1 ou 2, dans laquelle l'interleukine-2 est une interleukine-2 recombinante.

4. Composition selon la revendication 2, dans laquelle l'interleukine-2 humaine est une interleukine-2 humaine non glycosylée.

5. Composition selon la revendication 1, dans laquelle l'interleukine-2 a une activité spécifique de 20 000 à 80 000 unités/mg.

6. Composition selon la revendication 1, dans laquelle l'interleukine-2 est en une concentration de 1 à 80 000 unités/ml sous forme de solution aqueuse.

7. Composition selon la revendication 1, qui est exempte de sel.

8. Composition selon la revendication 1, qui comprend l'albumine sérique humaine.

9. Composition selon la revendication 8, dans laquelle l'albumine sérique humaine est en une concentration de 0,1 à 50 mg/ml sous forme de solution aqueuse.

10. Composition selon la revendication 1, qui comprend le composé réducteur.

11. Composition selon la revendication 10, dans laquelle le composé réducteur est un composé réducteur acide.

12. Composition selon la revendication 11, dans laquelle le composé réducteur acide est le glutathione, l'acide thioctique, la N-acétylcystéine, un acide thioalcanoïque comprenant de 1 à 7 atomes de carbone ou l'acide ascorbique.

13. Composition selon la revendication 10, dans laquelle le composé réducteur est en une concentration de 0,05 à 20 mg/ml sous forme de solution aqueuse.

14. Composition selon la revendication 1, qui comprend une combinaison d'albumine sérique humaine et du composé réducteur.

15. Composition selon la revendication 1, qui comprend en outre un acide aminé aliphatique monoaminé, un acide aminé cyclique, un monosaccharide, un alcool-sucre ou une combinaison de ceux-ci.

16. Composition selon la revendication 1, qui comprend en outre un acide aminé aliphatique monoaminé.

17. Composition selon la revendication 16, dans laquelle l'acide aminé aliphatique monoaminé est en une concentration de 5 à 50 mg/ml sous forme de solution aqueuse.

18. Composition selon la revendication 1, dans laquelle la composition est ajustée par un composé réducteur acide, un acide aminé acide, un acide minéral ou/et un tampon d'acide organique.

19. Composition selon la revendication 1, dans laquelle le pH est de 3 à 5,5.

20. Composition selon la revendication 1, qui est sous forme d'une solution aqueuse, d'un produit congelé ou d'un produit lyophilisé.

21. Composition selon la revendication 1, qui est sous la forme d'un produit lyophilisé.

22. Procédé de préparation d'une composition d'interleukine-2 comprenant de l'albumine sérique humaine, un composé réducteur ou une combinaison de celui-ci et qui est ajustée de façon à présenter, en solution, un pH de 3 à 6, selon lequel on incorpore de l'albumine sérique humaine, un composé réducteur ou une combinaison de celui-ci à une solution aqueuse d'interleukine-2 et on ajuste la solution de façon à ce qu'elle présente un pH de 3 à 6.

23. Procédé selon la revendication 22, selon lequel on congèle ensuite la solution formée pour obtenir un produit congelé.

24. procédé selon la revendication 22, selon lequel on lyophilise ensuite la solution formée pour obtenir un produit lyophilisé.

25. Procédé selon la revendication 22, selon lequel on ajuste la solution aqueuse au moyen d'un composé réducteur acide, d'un acide aminé acide, d'un acide minéral ou d'un tampon ou d'un acide organique.

26. Procédé selon la revendication 25, selon lequel on ajuste la solution aqueuse au moyen d'un acide minéral.

27. Procédé selon la revendication 25, selon lequel on ajuste la solution aqueuse au moyen du composé réducteur acide.

28. Composition d'interleukine-2 selon la revendication 1 pour son utilisation à la préparation d'un type nouveau d'agent anticancéreux ou d'un agent thérapeutique des déficits immunitaires.

Patentansprüche

1. Interleukin-2-Zusammensetzung, die Human-Serumalbumin, eine reduzierende Verbindung oder eine Kombination derselben umfaßt und so eingestellt ist, daß sie als Lösung einen pH-Wert von 3 bis 6 zeigt.

2. Zusammensetzung nach Anspruch 1, worin das Interleukin-2 Human-Interleukin-2 ist.

3. Zusammensetzung nach Anspruch 1 oder 2, worin das Interleukin-2 ein rekombinantes Interleukin-2 ist.

4. Zusammensetzung nach Anspruch 2, worin das Human-Interleukin-2 ein nicht-glycosyliertes Human-Interleukin-2 ist.

5. Zusammensetzung nach Anspruch 1, worin das Interleukin-2 eine spezifische Aktivität von 20 000 bis 80 000 Einheiten/mg hat.

6. Zusammensetzung nach Anspruch 1, worin das Interleukin-2 als wäßrige Lösung in einer Konzentration von 1 bis 80 000 Einheiten/ml vorliegt.

7. Zusammensetzung nach Anspruch 1, die salzfrei ist.

8. Zusammensetzung nach Anspruch 1, die das Human-Serumalbumin umfaßt.

9. Zusammensetzung nach Anspruch 8, in der das Human-Serumalbumin als wäßrige Lösung in einer Konzentration von 0,1 bis 50 mg/ml vorliegt.

10. Zusammensetzung nach Anspruch 1, die die reduzierende Verbindung umfaßt.

11. Zusammensetzung nach Anspruch 10, worin die reduzierende Verbindung eine saure reduzierende Verbindung ist.

12. Zusammensetzung nach Anspruch 11, worin die saure reduzierende Verbindung Glutathion, 6,8-Dithiooctansäure, N-Acetylcystein, Thioalkansäure mit 1 bis 7 Kohlenstoff-Atomen oder Ascorbinsäure ist.

13. Zusammensetzung nach Anspruch 10, worin die reduzierende Verbindung als wäßrige Lösung in einer Konzentration von 0,05 bis 20 mg/ml vorliegt.

14. Zusammensetzung nach Anspruch 1, die eine Kombination aus dem Human-Serumalbumin und der reduzierenden Verbindung umfaßt.

15. Zusammensetzung nach Anspruch 1, die weiterhin eine aliphatische Monoaminosäure, eine cyclische Aminosäure, ein Monosaccharid, einen Zuckeralkohol oder eine Kombination derselben umfaßt.

16. Zusammensetzung nach Anspruch 1, die weiterhin eine aliphatische Monoaminosäure umfaßt.

17. Zusammensetzung nach Anspruch 16, worin die aliphatische Monoaminosäure als wäßrige Lösung in einer Konzentration von 5 bis 50 mg/ml vorliegt.

18. Zusammensetzung nach Anspruch 1, worin die Zusammensetzung durch eine saure reduzierende Verbindung, eine saure Aminosäure, eine Mineralsäure oder/und einen Puffer einer organischen Säure eingestellt wird.

19. Zusammensetzung nach Anspruch 1, worin der pH-Wert 3 bis 5,5 beträgt.

20. Zusammensetzung nach Anspruch 1, die in Form einer wäßrigen Lösung, als eingefrorener Stoff oder als Lyophilisat vorliegt.

21. Zusammensetzung nach Anspruch 1, die in Form eines Lyophilisats vorliegt.

22. Verfahren zur Herstellung einer Interleukin-2-Zusammensetzung, die Human-Serumalbumin, eine reduzierende Verbindung oder eine Kombination derselben umfaßt und so eingestellt ist, daß sie als Lösung einen pH-Wert von 3 bis 6 zeigt, umfassend das Einbringen von Human-Serumalbumin, einer reduzierenden Verbindung oder einer Kombination derselben in eine wäßrige Lösung von Interleukin-2 und das Einstellen der Lösung, so daß sie einen pH-Wert von 3 bis 6 zeigt.

23. Verfahren nach Anspruch 22, worin die resultierende Lösung weiterhin eingefroren wird, um eine eingefrorene Zusammensetzung zu erhalten.

24. Verfahren nach Anspruch 22, worin die resultierende Lösung weiterhin lyophilisiert wird, um eine lyophilisierte Zusammensetzung zu erhalten.

25. Verfahren nach Anspruch 22, worin die wäßrige Lösung durch eine saure reduzierende Verbindung, eine saure Aminosäure, eine Mineralsäure oder einen Puffer oder eine organische Säure eingestellt wird.

26. Verfahren nach Anspruch 25, worin die wäßrige Lösung durch die Mineralsäure eingestellt wird.

27. Verfahren nach Anspruch 25, worin die wäßrige Lösung durch die saure reduzierende Verbindung eingestellt wird.

28. Interleukin-2-Zusammensetzung nach Anspruch 1 zur Verwendung bei der Herstellung eines neuen Typs eines Anti-Krebs-Mittels oder eines therapeutischen Mittels für einen Immunmangel.